CHEMISTRY.—*The destruction of hydrocyanic acid by prunase and the influence of sugars on the reaction.*[1] JAMES F. COUCH and REINHOLD R. BRIESE, U. S. Bureau of Animal Industry.

In the course of investigations being conducted in this laboratory on cyanogenesis in plants, it has become evident that the cyanogenetic enzyme is one of the factors that cause loss of freed HCN. In 1889 Tammann[2] observed that, when dilute solutions of HCN were treated with emulsin, 5 to 50 percent of the HCN is no longer detectable after 24 hours. This observation seems to have been generally forgotten. It may, however, explain the fact reported by Auld[3] that when amygdalin and emulsin react the resulting solution contains less HCN than is equivalent to the dextrose formed during the reaction. In the analysis of cyanogenetic plants such a loss of HCN may be very serious, especially if the HCN content of the plant be small and the enzyme very active.

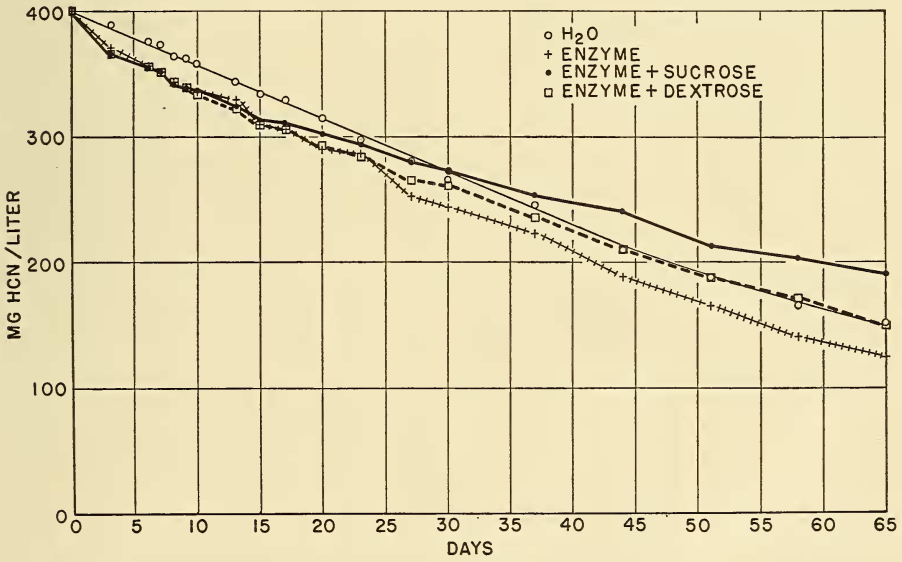

Fig. 1.—Graph of hydrocyanic-acid destruction in water and prunase solutions.

To obtain some quantitative data on this phenomenon a series of experiments was performed. Three liters of a water solution of HCN adjusted to contain 400 mg per liter were prepared. Four 750-cc portions were measured into 1-liter pyrex flasks. One portion was kept as a control. To the second portion was added 10 g of a crude

[1] Received February 21, 1939.
[2] Zeitschr. physik. Chem. 3: 25–37. 1889.
[3] Journ. Chem. Soc. T. 93: 1276–1281. 1908.

prunase preparation obtained from *Prunus serotina*. The preparation was made by extracting the cyanogenetic glucoside from fresh leaves with alcohol and drying the residue at room temperature. The powdered marc so obtained exhibited a strong activity toward crude prunasin. It gave 4.3 mg per 100 g HCN on analysis. The experimental results were corrected for the enzyme HCN.

Since there is some evidence that carbohydrates affect the rate of cyanogenesis in plants, a third portion was treated with 10 g of the enzyme preparation and 5 g of pure dextrose was added. A fourth portion was treated with 5 g of sucrose in addition to 10 g of the enzyme preparation. The four mixtures were stored in a dark place at $25° \pm 0.5°$ and 25-cc portions were withdrawn at intervals for analysis. The mixtures were well shaken several times a day. The results are plotted in Fig. 1. No attempt was made to adjust the mixtures to an optimum *p*H to avoid complication by possibly interfering substances. After 65 days the HCN content of the several lots was: 1, 151; 2, 129; 3, 155; and 4, 196 mg/liter. The curves show that in the presence of prunase HCN disappears more rapidly than in dilute water solution. Dextrose had little effect during the first third of the experiment but neutralized the effect of the prunase to a large extent thereafter. For the first fourth of the experiment sucrose likewise appeared to exert little effect. Then the rate of loss of HCN began to diminish and after the twenty-seventh day the sucrose mixture always contained more HCN than any of the other three.

Although the order of the reaction is not settled, it is of interest to calculate the reaction coefficient. In the absence of definite information to the contrary the monomolecular reaction constant was determined for each series and for the entire period of 65 days. These are stated in the last column of Table 1. The figures indicate the acceleration of the decomposition by prunase, the retardation by sucrose, and a negligible net effect of dextrose.

TABLE 1.—DESTRUCTION OF HYDROCYANIC ACID IN WATER AND PRUNASE SOLUTIONS

Solution	*p*H at end	HCN lost	*k*
		Percent	
Control	3.16	62.2	452×10^{-8}
Enzyme	4.10	67.7	523
Enzyme plus dextrose	3.80	61.2	445
Enzyme plus sucrose	3.82	51.0	320

It was thought that the retarding action of sucrose beginning only some 17 days after the start of the experiment might be due to inversion of the sucrose known to take place in aqueous solutions and

that the levulose as formed was actually the retarding compound. To test this hypothesis under actual plant conditions, dextrose, sucrose, and levulose were added to mixtures of ground fresh cyanogenetic plants with water and mercuric chloride as a preservative,[4] which were allowed to macerate for various times at 25°. At the end of four weeks to six months the stored mixtures were analyzed for HCN and compared with a control sample to which sugars had not been added. The latter, however, contained small amounts of their natural carbohydrates, dextrose in *Prunus serotina* and both dextrose and sucrose in the sorghums. The results appear in Table 2. There

TABLE 2.—EFFECT OF SUGAR ADDED TO CYANOGENETIC PLANT MIXTURES

Date	Plant	Weight of plant	Weight of sugar added	$HgCl_2$	Period	HCN recovered after adding—			
						Dextrose	Sucrose	Levulose	Control
1938		*g*	*g*	*Per cent*	*Weeks*	*mg/100 g*[1]	*mg/100 g*[1]	*mg/100 g*[1]	*mg/100 g*[1]
May 22	Prunus serotina	25	5	2	26	101	100		102
May 22	Prunus serotina	25	5	2	4	98	94		99
June 28	Prunus serotina	25	5	2	4		129		129
July 18	Sorghum vulgare var. hegari	50	2.5	2	8	38	38	40	39
July 25	Sorghum vulgare var. hegari	50	2.5	2	8			35	37
July 27	Sorghum vulgare var. Sharon kafir	50	5	2	8			14	14
July 28	Sorghum vulgare var. hegari	50	5	2	8			28	28
Aug. 22	Sorghum vulgare var. hegari	50	5	3	4	30	33	36	34

[1] mg per 100 g of plant.

are no very significant differences between the figures for the various sugars and the controls. Levulose appeared to yield slightly higher results than dextrose or sucrose but did not differ significantly from the controls. The differences observed between these experiments and those reported in Table 1 may be ascribed to the presence of mercuric chloride which combines with the HCN liberated by enzymolysis of the glucoside and indicate that any direct action that carbohydrates may exert in cyanogenetic mixtures is on the freed HCN rather than on the enzymolysis. In the absence of a preservative like mercuric chloride, prunase is capable of accelerating the decomposition of HCN in water solution. Dextrose and sucrose neutralize this action but only after some time has elapsed.

[4] Briese, R. R., and Couch, J. F. Journ. Agr. Res. **57**: 81–107. 1938.